Reticulocytosis, Hypochromia, and Microcytosis: An Unusual Presentation of the Preleukemic Syndrome


Two patients exhibiting a highly unusual preleukemic syndrome with marked reticulocytosis, hypochromia, and microcytosis are reported. This red cell phenotype has been investigated by means of HbF, HbA2, and i-antigen activity dosages. Immunofluorescence labeling of F cells, reticulocyte survival, globin chain synthesis, and electron microscopy study. The marked reticulocytosis is explained by a delayed disappearance of the reticulum. Serum iron is normal, and a thalassemic syndrome is excluded because of a balanced α/non-α globin chain synthesis. Electron microscopy studies are consistent with a defect in iron uptake by erythroid cells. All the hematologic data and investigations are similar to those observed for the Belgrade laboratory rat. It is hypothesized that the low expression of HbF and i-Ag associated with microcytosis are related to a prolongation of erythroid maturation as reflected by abnormal reticulocyte survival.

Preleukemia is a recognizable syndrome of hematopoietic dysfunction that may precede the classical findings of acute myelogenous leukemia. It is usually characterized by ineffective hematopoiesis with peripheral pancytopenia and a hypercellular bone marrow. A substantial proportion of patients with preleukemia die of complications related to leukopenia and thrombocytopenia; some patients however develop frank acute myelogenous leukemia several months or years after the diagnosis of preleukemia.

Several abnormalities have been observed in the erythroid lineage such as: (A) morphological abnormalities of the nucleus and of the cytoplasm of normoblasts with iron granules often present in the mitochondria; (B) proliferation defects determining an accumulation of erythroblasts in G1 phase of the cell cycle; (C) increased corpuscular volume, which is a very common finding; (D) several abnormalities of erythrocyte enzymes of which the most frequent are a decrease in pyruvate kinase and cholinesterase activity; (E) increase in fetal hemoglobin concentration; (F) alterations in blood group antigen expression: increase of erythrocyte i-antigen and a decrease in A1 and H substance; (G) imbalance α/β globin chain synthesis with acquired HbH. None of these abnormalities are constant findings, but they may contribute to a better insight into the differentiation pattern of the erythroid lineage in preleukemia.

In the present study we report two patients exhibiting unusual laboratory findings associated with a preleukemic condition; both patients exhibited a marked and constant reticulocytosis, hypochromia, and microcytosis. These laboratory findings suggested that reticulocytosis could be related to an abnormal delay in reticulocyte maturation, while microcytosis and hypochromia could be ascribed to a maturation defect related to iron uptake by red cells.

### Table 1. Hematologic Data

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>December 79</td>
<td>December 73</td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>8.9</td>
</tr>
<tr>
<td>MCV (μl)</td>
<td>72</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>25.8</td>
</tr>
<tr>
<td>Reticulocytes/μl</td>
<td>960,000</td>
</tr>
<tr>
<td>Erythroblasts/μl</td>
<td>1,800</td>
</tr>
<tr>
<td>Platelets/μl</td>
<td>25,000</td>
</tr>
<tr>
<td>Leukocytes/μl</td>
<td>5,400</td>
</tr>
<tr>
<td>percent neutrophils</td>
<td>28</td>
</tr>
<tr>
<td>percent blasts</td>
<td>19</td>
</tr>
<tr>
<td>percent erythroblast</td>
<td>15</td>
</tr>
<tr>
<td>Nonconjugated bilirubin (mg/dl)</td>
<td>1.6</td>
</tr>
<tr>
<td>Serum iron (μg/dl)</td>
<td>85</td>
</tr>
<tr>
<td>Serum ferritin (pg/ml)</td>
<td>840</td>
</tr>
<tr>
<td><strong>Bone marrow</strong></td>
<td></td>
</tr>
<tr>
<td>Myeloblasts</td>
<td>19</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>10</td>
</tr>
<tr>
<td>Myelocytes</td>
<td>13</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>7</td>
</tr>
<tr>
<td>Mature granulocytes</td>
<td>10</td>
</tr>
<tr>
<td>Erythroid cells</td>
<td>38</td>
</tr>
<tr>
<td>No sideroblast</td>
<td>No sideroblast</td>
</tr>
</tbody>
</table>

Case 1

A 70-yr-old woman was admitted to Raymond Poincaré Hospital (Garches) in October 1979 with prolonged fever and ecchymoses. Two years before she had complained of lethargy and fever. Because of the presence of an hepatomegaly, a laparoscopy with hepatic biopsy was performed that revealed an epithelioid granuloma, possibly tuberculosis. The hematologic findings were normal. She received Isoniazide, ethambutol, and streptomycin for 1 yr, with a good effect on the general condition that returned to normal within 3 mo.

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Physical examination in October 1979 was unremarkable except for the presence of a splenomegaly. Her hemoglobin level was 8.10 g/dl with MCV 76 Cmu, MCHC 25.8 g/dl, and the reticulocyte count was 924,000/μl. The white blood cell count was 5400 with 26% neutrophils, 19% myeloblasts, and 15 erythroblasts per 100 white blood cells. Serum iron was normal at 80 μg/dl, and serum ferritin concentration was increased (600 pg/ml). Bone marrow examination revealed a marked cellular hyperplasia with an excess of myeloblasts (19%) and promyelocytes (14%). Hemosiderin was not detected in erythroblasts. The bone marrow picture was consistent with a diagnosis of refractory anemia with excess of myeloblasts. The red cell lifespan as estimated by 51Cr was reduced (12 days) (25 days for normal controls).

In December 1979, she was readmitted to the hospital. The hematologic data are summarized in Table 1. The patient’s condition remained unchanged until March 1980 (Fig. 1), when she died from intracranial hemorrhage.

This observation led us to consider the hematologic findings observed 7 yr ago in a similar case.

**Case 2**

A 64-yr-old woman was found in June 1973 to have an anemia: Hb 9.19 g/dl. She was subsequently admitted in Henri Mondor Hospital (Créteil) in July 1973. Investigations revealed the following results: Hb 7.9 g/dl, MCV 80 Cmu, white cell count 2200/μl with 59% neutrophils, platelet count 24,000/μl, and reticulocyte count 173,000/μl; unconjugated bilirubin was 2.4 mg/dl and serum iron 120 μg/dl. Bone marrow aspiration revealed a marked hyperplasia of both myeloid and erythroid series with an increase in myeloblasts (20%) and promyelocytes (12%). Hemosiderin was not detected in erythroblasts after prussian blue staining, but marrow hemosiderin stores in macrophages were highly increased. A diagnosis of refractory anemia with excess of myeloblast was made, she did not receive any chemotherapy.

In September 1973, the reticulocyte count was 680,000/μl, in October 1973, 910,000/μl. In December 1973, she was readmitted to the hospital because of bleeding; physical examination revealed a splenomegaly. Hematologic data are summarized in Table 1. Subsequent follow-up showed no consistent change, and reticulocyte counts were always above 1,000,000/μl.

In February 1974, an attempt was made to reverse the patient’s thrombocytopenia and bleeding symptoms by initiating a regimen of daunorubicin and cytosine arabinoside. No response to therapy occurred and she died after a 12-day long period of aplasia, with persistence of myeloblastic infiltration in the bone marrow.

**MATERIALS AND METHODS**

**HbF and HbA2, Dosages, F Cells**

HbF was determined according to the method of Betke. Measurement of HbA2 was performed by microchromatography. The proportion of F cells was determined by immunofluorescence.
using an anti-HbF rabbit IgG, prepared according to the technique of Wood\textsuperscript{26} with slight modifications.\textsuperscript{27}

**Reticulocyte Survival**

Fresh venous blood, collected in ACD, was diluted 10 times with medium (Eurobio, Paris, France) and then incubated in a Corning flask at 37°C in the presence of 5% CO\textsubscript{2}. At prefixed times, 0.5-ml aliquots were removed and the proportion of reticulocytes was determined by new methylene blue staining.\textsuperscript{22} For each sample, at least 2000 RBCs were counted.

**Hemoglobin Synthesis**

Red blood cells were washed 3 times in NCTC-109 medium leucine-free (Eurobio, Paris, France) and then incubated for 2 hr at 37°C in the presence of 200 μCi 3H-leucine (Euratom, Commissariat à l'Energie Atomique, Saclay, France). At the end of the incubation period, white blood cells were removed by filtration on cellulose columns, as previously reported.\textsuperscript{23}

Globin chains were separated by chromatography on carboxymethylcellulose in 8 M urea, following the method of Clegg and Weatherall,\textsuperscript{24} slightly modified by Testa et al.\textsuperscript{25} In order to investigate the effect of hemin on hemoglobin synthesis, hemin was added to the incubation medium. Hemin was dissolved in distilled water at alkaline pH and then added to the incubation medium to give a final concentration of 100 μM. At prefixed times during the incubation aliquots were removed and the TCA precipitable radioactivity was determined, as previously described.\textsuperscript{26} In one experiment, iron-saturated human transferrin (Sigma, St. Louis, Mo.) was added to the incubation medium.

**Blood Group Antigens**

The expression of i, I, H, A, and B antigens was investigated by a quantitative agglutination assay as previously described.\textsuperscript{27}

**Electron Microscopy Studies**

Red blood cells and circulating erythroblasts were immersed in 1.25% glutaraldehyde in 0.1 M phosphate buffer and fixed for 30 min at 4°C. The fragments were then postfixed for 30 min at 4°C in 1% osmium tetroxide solution followed by dehydration in a series of alcohol and propylene oxide and embedded in Epon. Thin sections were obtained with a Reichert OM U\textsubscript{2} microtome and were doubly stained with uranyl acetate and lead citrate. They were examined in a Philips EM 300 electron microscope.

**RESULTS**

In order to explain the marked reticulocytosis, the in vitro reticulocyte survival was studied. The results of this study are reported in Fig. 2. In the controls (one patient with β-thalassemia intermedia and one cord blood specimen) 50% of the reticulocytes disappeared in 10–12 hr as compared to more than 72 hr in patient 1. These reticulocytes were still functionally active after 72 hr of incubation, as shown by their capacity to synthesize hemoglobin at a high rate as demonstrated by 3H-leucine incorporation. The course of some hematologic parameters is shown in Fig. 1; the reticulocyte count, MCV, and MCHC remained abnormal.

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Fig. 2. Reticulocytes survival in patient 1 as compared to two controls (β-thalassemia intermedia and cord blood). Half-life in patient 1 was higher than 72 hr, while in the two controls it was about 12 hr. More recently, three other controls (autoimmune hemolytic anemia, β-thalassemia, and myelofibrosis) have shown a half-life of less than 24 hr.

Fig. 3. Acidophilic erythroblast exhibiting no iron micelles in mitochondria and absence of ferritin in a vesicle of rhophoeocytosis (arrow).
at several months interval. Electron microscopy studies in patient 1 showed no figures of iron micelles in mitochondria of erythroid cells, confirming the Perls finding of absence of abnormal sideroblasts. Accurate analysis of rhophocytosis vesicles on the surface of erythroblasts revealed the complete absence of ferritin (Fig. 3), suggesting a reduction of iron uptake by these erythroid cells.

Study of $^3$H-leucine incorporation into hemoglobin in the absence or in the presence of 100 $\mu$M hemin only showed a slight stimulation by hemin addition (1.3 times at 60 min of incubation). The addition of iron-saturated transferrin to the incubation medium did not increase the incorporation of $^3$H-leucine into hemoglobin. The globin chain ratio was in the normal range, 0.88 (0.9–1.1 for normal controls), and was unmodified by hemin addition.

Hemoglobin analysis revealed a marked reduction in the level of HbA$_2$; in addition, HbF was undetectable by Betke’s technique. Immunofluorescent studies by a specific anti-HbF antibody showed that in patient 1 only a small proportion of erythrocytes had HbF (0.1%).

Erythrocytes from the two patients were not agglutinated by a specific anti-i serum, while a high proportion of preleukemic patients exhibited a high i-agglutinability (Fig. 4).

Patient 1 belonged to blood group B, and she exhibited an increase of I-antigen expression associated with a decrease of H-antigen expression as compared
to normal subjects and to other preleukemic patients (Fig. 5). Patient 2, who was blood group A1, exhibited a slightly increased expression of I-antigen associated with a decrease in A1 antigen expression with respect to normal controls.

DISCUSSION

The two patients reported here exhibited a preleukemia syndrome of the RAEM type (refractory anemia with excess of myeloblasts) with some unusual features.

First, they exhibited a marked reticulocytosis. A slight reticulocytosis occurs in 35% or 50% of patients with preleukemia, but it rarely exceeds 10% of the red cells. The reticulocytosis in previous studies was attributed to a hemolytic process and in a single case to delayed maturation of reticulocytes. Our studies suggest that at least for patient 1, the second mechanism is mainly operating: indeed, the in vitro reticulocyte survival showed a delayed disappearance of the reticulum. This delay in maturation could be the consequence either of an abnormal ribonucleoproteic complex or of a deficiency of the ribonucleasic system responsible for the digestion of the reticulum. Lofters et al. have previously suggested that the first mechanism could operate, since they observed an abnormal α/β globin chain ratio in a patient in which preleukemia was associated to marked reticulocytosis. This was not the case in patient 1 who exhibited a balanced globin chain ratio.
Secondly, the red cells were microcystic. Microcytosis is exceptionally observed in preleukemia as well as in acute myelogenous leukemia.

In the two patients, microcytosis could not be related to iron deficiency since their plasma iron as well as their ferritin levels were increased. In addition, microcytosis was associated to hypocromia. Laboratory findings as well as studies of Hb synthesis into reticulocytes in the presence of heme have demonstrated that there was not an important impairment in heme synthesis. Furthermore, the existence of a thalassemic syndrome was excluded by globin chain studies. Assemic syndrome was excluded by globin chain synthesis, which showed a balanced α/α ratio. Thus, the main known cause of microcytosis and hypocromia can be excluded in our patients.

Patient 1 had had a 1-yr regimen of isoniazid 2 yr prior to our study. Thus, the hypothesis of an impairment in pyridoxine metabolism could be raised. However, because of the absence of sideroblast in the bone marrow and the lack of stimulation of hemoglobin synthesis in vitro after addition of heme, this hypothesis can be ruled out.

An alternative mechanism could be a defect in iron uptake by erythroid cells, since electron microscopy studies demonstrated no ferritin at the level of rhopheocytotic vesicles. This finding underlines a defect in iron uptake, i.e., a defect in transferrin receptor expression, on the surface of erythroid cells.

This suggestion is further supported by the comparison of the hematologic findings in our patients with the Belgrade laboratory rat. This strain of rat exhibits an anemia characterized by hypocromia, microcytosis, and marked reticulocytosis; serum iron is increased and sideroblasts are absent in bone marrow. Several studies have shown that the globin chain ratio is balanced, and the iron uptake by reticulocytes in vitro is markedly reduced. This last phenomenon was due not to a defect in transferrin binding but to a block in iron internalization. Thus, the phenotype exhibited by our patients is very similar to that reported in the Belgrade anemic rat; this finding suggests that a single defect in cellular iron metabolism may be responsible for a complex phenotype in red cells. This defect in iron metabolism could be either the consequence of the preleukemic state or a condition predisposing to leukemia.

An additional unusual finding in these two patients is represented by the data concerning HbA2, HBF, and i and I antigens. It has been recently demonstrated in vitro that HBF synthesis and i and I expression are regulated by the process of differentiation, including early and late phases. There is also increasing evidence that erythroid maturation also modifies the expression of H and A antigens. In preleukemic states, there are striking changes in the pattern of erythroid differentiation, typically allowing for an acceleration of the maturation steps of the erythron. An increase in HBF level and i antigen expression is usually observed. These abnormalities are correlated with macrocytosis. In the two patients, an inverse phenomenon may operate, since concentration of HbA2, HBF, and expression of i-antigen are decreased while a microcytosis is present. It can be hypothesized that these different changes are directly related to a prolongation in the erythroid maturation period. This hypothesis is strengthened by the abnormal length of reticulocyte maturation.

All the present laboratory investigations underline that a single defect, here in the cellular metabolism of iron, may be responsible for a complex erythroid phenotype. This erythroid phenotype cannot be related to the defects in iron metabolism usually observed.

It can thus be suggested that the modifications of the phenotype occurring in the red cells during preleukemia can be essentially the consequence of a single change in the differentiation process of the erythron.

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